Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.6, No.23, 2016

Review on Invitro Regeneration of Some Selected Grapevines (Vitis vinifera L.) Cultivars from Shoot and Leaf Culture

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Abstract
Invitro regeneration is aseptic culture of cells, tissues, organs or whole plants under controlled nutritional and environmental conditions. Grapevine (*Vitis vinifera* L.) is one of the most widely distributed fruit crops in the world. Conventional method of grapevine propagation is time consuming and allows disease transmission. A planted grape vine needs four to five years to be a propagation material by cutting, due to its long juvenility period. Therefore, the establishment of efficient *in vitro* regeneration is too much needed. The optimal levels of growth regulators and light conditions on callus induction and organogenesis of *in-vitro* cultured grapevine were assessed. Accordingly, maximum calluses and shoots were produced by using medium supplemented with different concentrations of growth regulators (BAP, IBA, TDZ and NAA) as alone or in combinations. For successful production of propagules, the occurrence and effect of *in vitro* shoot vetrification /hyperhydricity/were assessed. From the tested different concentrations of BAP and agar, the best mean numbers of vetrification free shoots were obtained at 0.5 mg/l BAP in 7.5g/l agar for both cheninblanc and canonannon cultivars.
Keywords: Organogenesis, Hyperhydricity, growth regulators, callus induction, Grapevine

1. Introduction

Grapevine (*Vitisvinifera*L.) is one of the most widely distributed fruit crop in the world. It grows from temperate to tropical regions, but most vineyards are planted in areas with temperate climates. Wild grapevines occur primarily in the Northern Hemisphere, especially in the temperate zone in Asia, North America, Central America, and Northwest of South America in the Andes chain in Colombia and Venezuela. This distribution highly deals with historical connections with the development of human culture (Patrice*et al.,* 2006).

It is believed that grape cultivation originated near Caspian Sea in Russia that spread westward to Europe and American continents and eastward towards Iran and Afghanistan (Richard *et al*., 2010). However, *Vitisvinifera* originated in the regions between and south of the Caspian Black Seas in Asia and has been distributed from region to region in all temperate climates (Krongjai, 2005). According to Patrice *et al*. (2006), *V. vinifera* is highly distributed and produces over 90% of the world's grapes, which are either pure *V.vinifera*orhybridized with one or more American species today. About 85% of the grapes in the United States are derived from pure *vinifera*varieties (Krongjai, 2005). Thus, most important varieties of pure species in North America used for fruiting are from the varieties of *V.labrusca* (Concord, Niagara*), V. rotundifolia*(Scuppernong, Eden), and *V. rupestris* (Rupestris St. George-used mainly as phylloxera-resistant rootstocks). This old world species, *V. vinifera*, is the grape of antiquity often mentioned in the Bible. The main product, wine, was considered divine, a drink of the gods (Patrice*et al*., 2006).

Other Mediterranean cultures considered that 'the wine sprang from the blood of humans who had fought the gods' and wine has always had a major role in the way of life of Mediterranean people (Patrice*et al*., 2006). Cultivated grapevines now exist on every continent on earth wherever the climatic conditions are favorable except for Antarctica. In 2001, global vineyard acreage was 7.9 million hectares. However, when compared with the period 1997-2000, the most significant vineyard acreage increased in 2001. Their distribution is different from country to country: China (57.9%), Australia (31.6%), New Zealand (28.7%), Chile (17.0%), United States (9.7%) and Iran (8.5%). In 2001, Some African countries such as Algeria, Egypt, Libya, Tunisia and South Africa have also participated in production of grape. In case of Ethiopia, wineries are importing about 300 tons of grape production per year in the form of dried raisin, grape juice concentrates, natural wine extracts and citric acid (Patrice*et al*., 2006). The conventional method of grapevine propagation is time consuming and allows disease transmission. Juvenility is one of the principal naturally occurring problems hindering grapevine production (Rossel, 1992; Winkler (1974). Although the grapevine is the third most important fruit crop in the world after banana and citrus, today the need for grapevine fruit is increasing (Richard *et al.,* 2010). Typically, this happened because of increase in the number of wine industries and more demand for fresh and dried fruits (Fayek *et al.,* 2009). According to Aazami *et al*. (2010), genetic improvement of the classic cultivars in order to obtain high quality wine and table grape varieties through conventional hybridization methods does not appear to be enough. Therefore, the non-conventional methods such as micropropagation and plant regeneration systems could be used.

Micropropagation in grapevine was first performed by "*in vitro*" culture of micro-cuttings (Aazami*et al*., 2010) to propagate the varieties. However, more recently, introduction of bud proliferation has been shown to

provide an alternative pathway to grapevine micropropagation (Aazami*et al*., 2010). However, the developed technique should result in rapid clonal multiplication and uniform plants, normal yield and healthy plants. In addition, the *in vitro* vegetative multiplication techniques should be designed in the form of unchanging genetic makeup, basic biological, physiological, and horticultural characteristics (Chee *et al.,* 1984).

Tissue culture and the commercial production of plantlets in different parts of the world are limited to a few outstanding regional cultivars. Even though micropropagation represents an efficient method of plant regeneration and rapid propagation of any valuable genotype obtained by nonconventional methods, to do *in vitro* selection and genetic transformation need the *in vitro* regenerated plantlets of the varieties (Pe´ros *et al.,* 1998).

2. Literature review

2.1. Structure and growth stage of grapevine

Like most other plants, the grapevine has a predictable cycle of growth. Life cycle of the grapevine can be categorized under certain stages, depending upon the growth pattern of vitis plants. Bud break, flowering stage, the fruit stage, and harvesting stages are the complete set of life cycle of grapevine (Krongjai, 2005). From commercial viticulture perspective, nearly all grape varieties are propagated through stem cutting, layering and grafting in most parts of the world. However, this increases the susceptibility of cultivated varieties to disease causing agents (microbes, mites, insects, nematodes, fungi, bacteria, viruses and more importantly *Phylloxera)* (Alizadeh *et al*., 2010).

2.2. Invitro regeneration of grapevine

The micropropagation of grape has been reported previously by many authors. Thus, use of *in vitro* techniques for propagation of various *Vitisvinifera* cultivars has been well-documented (Alizadeh *et al.,* 2010*)*. According to Rossel (1992), the expansion of vineyard cannot be achieved without the pre-establishment of techniques that make adequate amount of planting materials available within short time. Hence, it is better to develop the technique for large-scale production. This might need to develop an effective technique based on the plant species and cultural conditions within the small size place and then to field system.

Despite years of investigation, the application of tissue culture techniques in the grape-growing industry is still limited (Pe´ros*et al.,* 1998). Hence, different cost effective protocols for organogenesis should be developed (Deore and Johnson, 2008). Thus, an establishment of such efficient protocol for high-frequency direct regeneration of plantlets from leaf explants of *Vitisvinifera* has a vital role in the analysis of genetic material and mass propagation of plants in short period of time. Accordingly, effective mass propagation of grapevine from shoot tips of some grapevine cultivars were successful on different concentrations of growth regulators (Beza Kinfe, 2010)

Table 1: Mean number of shoot at different BAP concentration

No of explant	BAP(mg/l)	Mean number of shoots/explant		
		Ugni blanc	Chenin blanc	canonannon
25	0	1.0c	1.0c	1.0c
25	0.25	2.5bc	2.4b	2.6b
25	0.5	5.3a	5.0b	5.6a
25	1	2.8bc	2.9b	2.8b
25	2	3.4ab	3.4b	3.4b
25	3	2.8bc	3.2b	3.4b
25	4	2.8bc	3.3b	3.0b

Source:(BezaKinfe, 2010 book)

On other hand, the affectivity of growth regulators had been identified for Soltanin' and 'Sahebi' cultivars (Aazami MA., 2010).

Table2. Effects of different hormonal treatments on "in vitro" shoot production in the 2 grape cultivars.

F Factors		Shoots per apex
Culture medium	A	1.6d
	B	3.8b
	C	5.4a
	D	2.5c
Cultivars	'Soltanin'	4.1a
	'Sahebi'	3.8a

A (1mgl^{-1}), B 1.5mgl^{-1}), C (1mgl^{-1} IBA +15mgl^{-1} BA) and D (1mgl-^{1}TDZ)

Source: (Aazami MA., 2010, article).

2.3. Organogenesis of Grapevine

The organogenesis is a biotechnological tool used for obtaining mass production of mother plant with high quality of health (Betton *et al.,* 2015). The explants can be grown into whole plant or produce callus. The produced callus can be utilized to regenerate plantlets or to extract or to manipulate some primary and secondary metabolites (Pande and Gupta, 2013). Plant mass production can be affected by several factors such as light, temperature, plant varieties, and type of explant, components of media, sources and orientation of explants (Kumar and Raddy, 2011).

Temperature influences the various physiological processes, such as respiration and photosynthesis, is well known and it is not surprising that it profoundly influences plant tissue culture and micro-propagation. The most common culture temperature range has been between 20°C and 27°C, but optimal temperatures vary widely, depending on genotype (Kumar and Raddy, 2011). Most of times, the optimal shoot proliferations of grapevine were reported when, both hormones (Cytokines and auxins) were combined. For instance for *Muscat of Alexandria cv* maximum number of proliferated shoots was obtained on MS medium containing 3.0 mg/l BAP + 0.2 mg/l NAA. Similarly, the best shoot inductions were recorded at 2.0 mg/l BAP + 0.1mg/l IAA for Canonnanon and Cheninblanc cultivars (Abido *et al*., 2013; Fikadu Kumsa, 2011).

According toAzami (2010), different combinations of growth regulators (1.5 mgl-1 BA), C (1 mgl-1 IBA+ 1.5 mgl-1 BA) were produced the best shoot for"*Soltanin*"and "*Sahebi* cultivars from merestem. Grapevine (*Vitisvinifera*L.) is one of the most valuable genotype mostly obtained by conventional methods. So developing *in vitro* propagation of grapes was not only for the wine industry, but also due to the demand for fresh and dried fruit (Abido, 2013). Despite years of investigation, the application of tissue culture techniques in the grape-growing industry is still limited (Pe´ros*et al.,* 1998). Hence, different cost effective protocols for mass propagation should be developed (Deore and Johnson, 2008). Beside the micro-propagations, an establishment of efficient protocol for high-frequency of indirect regeneration of plantlets is so much needed. Even though the indirect regeneration of grapevine were not success with different cultivars of grapevine, its reported that shoot were initiated from callus of *'canonannon' cultivar at 0.5mg/l BAP+1mg/l IBA at 35 days, after culture (FikaduKumsa, 2016).*

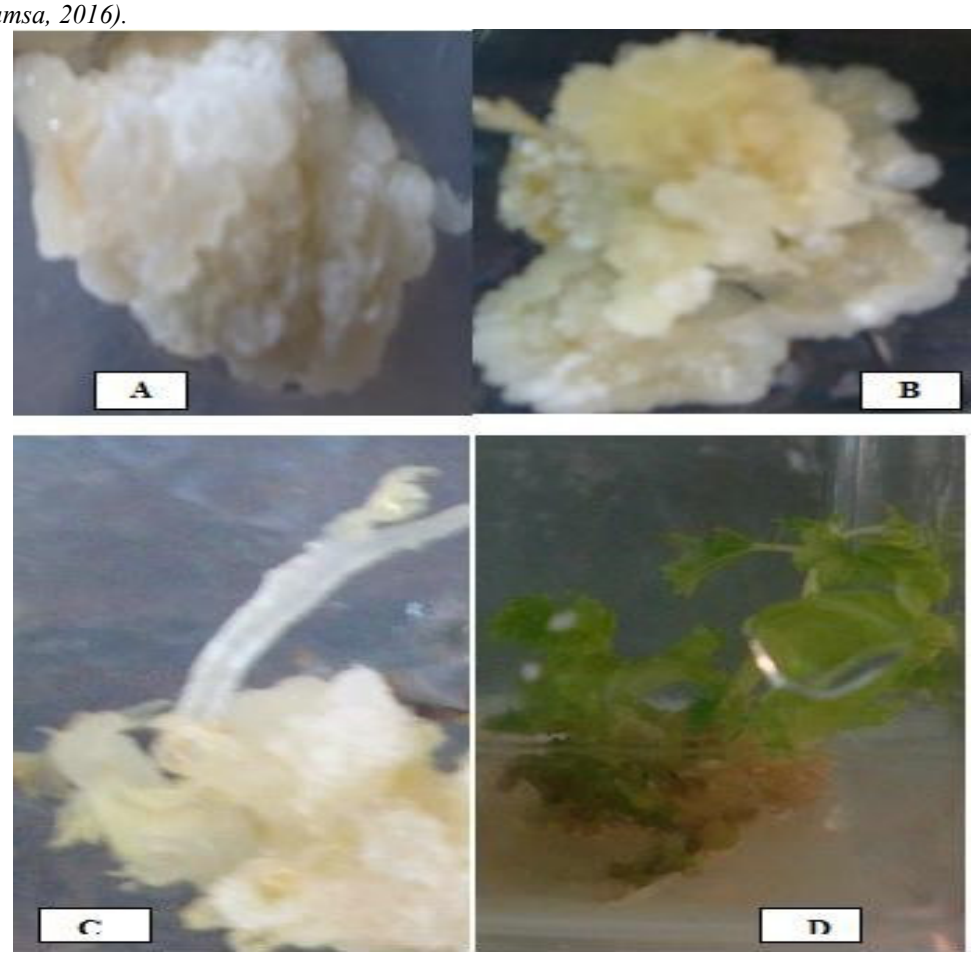

Figure 1:*Induced shoots of* **'***canonannon' cultivar from cultured callus on medium supplemented by 0.5mg/l BAP+1mg/l IBA at 35 days, after culture*

Source: Fikadu Kumsa, 2016 book

3. Grapevine Vetrification

Hyperhydricity of micropropagated shoots also known as vetrification, unquestionably results from growth and culture conditions, exposed to stressing factors, wounding, and using of soft culture media. Generally, of high ionic strength, rich in nitrogen and growth regulators in special balance in a humid and gaseous confined atmosphere can create vetrification (Kevers *et al.,* 2004). Hyperhydricity affecting herbaceous as well as woody shoots during their *in-vitro* vegetative propagations. The vetrified shoots appear turgid, watery at their surface, sometimes less green and easily breakable. Vetrified shoots root poorly when they do. They fit a problem of survive at acclimatization steps. Stems of vetrified plantlets are broad and thick in diameter. Nodes are short. Leaves are thick and elongated (Kevers*et al.,* 2004).

The hyperhydricity is also characterized by an excessive accumulation of water which is apparently associated to cellular oxidative stress. It also, gives place to a number of morphological, physiological and anatomical abnormalities. This condition is most likely to develop in vegetative materials grown *in vitro (*Osuna *et al*., 2011). In most micropropagation laboratories, vetrification is very serious problem most face of their cultures and many tissue cultures have focused their efforts on practical means of avoiding vetrification (Kevers*et al.,* 2004).

Vetrification affects the survival and quality of micro propagated plants. It highly affects the leaf. The thoughtful of fundamental mechanism of *in vitro* controlling vetrification can produce more efficient micropropagation (Rasco and Patena, 1997). As, vetrification of shoots appear during the multiplication stages, Reductions of vetrification*in vitro* result in increment of shoot numbers. There are a number of mechanisms used to reduce vetrification: can be reduced by aeration of culture volume and changing of the concentration of growth regulators (Sharma and Mohan, 2006). In another way, an effective procedure for obtaining healthy shoots from *in vitro* culture of propagates was ventilating the culture vessels (Laia *et al*., 2005).

Liquid and low agar media also one causing agent of vetrification as it induced cellulose formation along with induced and disoriented cellulose biosynthesis which is manifested in non-functional guard cells. Mal-functioning stomata in addition affect the cuticle contributed to increased transpiration and desiccation of *in vitro* formed leaves. Thus, agar should not be considered simply as a means of solidifying culture media: In general, the concentrations of agar affect the chemical and physical characteristics of a culture medium (Ziv, 1991). Though it is known that, grapevine (*Vitisvinifera*L.) is one of the most widely distributed fruit crop of the world, today the need for grapevine fruit is increasing (Richard *et al*., 2010). This happened because of increase in the number of wine industries and more demand for fresh and dried fruits (Fayek *et al*., 20090. So to fit the demand for grape a healthy micro-propagation is too much needed (Patrice *et al*., 2006). A vetrification is a serious problem during the *in vitro* propagation of grapevine. The problem of vetrification on micropropagation of the grapevinehas been reported (Alizadeha et al., 2010; BezaKinfe , 2010) but there are no reports which mention decreases of vetrification. There was study which assesses the effect of types of phyto-hormones and agar concentration on vetrification of 'Canonannon' and Cheninblanc' cultivars (Fikadu Kumsa and Tiliye Feyissa, 2016).

Table 3: Effect of agar and BAP on normal and vetrified shoots of grapevine at 3 weeks after culturing

		'Canonannon'		'Cheninblanc'	
Agar (g/l)	**BAP (mg/l)**	Mean no of normal shoots/explant	Mean no ofvetrified shoots/explant	Mean no of normal shoots/explant	Mean no ofvetrified shoots/explant
0.0	0.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
6.0	0.5	2.5± 0.8^{b}	2.5± 0.8^{b}	2.0± 0.1^{c}	2.0± 0.1^{c}
6.0	1.0	1.0± 0.6^{c}	1.0± 0.6^{c}	0.7± 0.2^{c}	0.7± 0.2^{c}
6.0	1.5	2.2± 0.3^{c}	2.2± 0.3^{c}	2.1± 0.8^{c}	2.1± 0.8^{c}
6.0	2.0	1.5± 0.2^{c}	1.5± 0.2^{c}	1.3± 0.6^{c}	1.3± 0.6^{c}
6.0	2.5	1.0± 0.1^{c}	1.0± 0.1^{c}	0.8± 0.5^{c}	0.8± 0.5^{c}
6.5	0.5	2.9± 0.5^{b}	2.5± 0.5^{b}	2.3± 0.4^{c}	2.3± 0.4^{c}
6.5	1.0	2.0± 0.8^{c}	2.0± 0.8^{c}	1.6± 0.3^{c}	1.6± 0.3^{c}
6.5	1.5	2.2± 0.2^{c}	2.2± 0.2^{c}	2.1± 0.1^{c}	2.1± 0.1^{c}
6.5	2.0	1.2± 0.1^{c}	1.2± 0.1^{c}	1.2± 0.8^{c}	1.2± 0.8^{c}
6.5	2.5	2.8± 0.7^{b}	2.8± 0.7^{b}	2.4± 0.9^{c}	2.5± 0.9^{b}
7.0	0.5	3.0± 0.9^{ab}	0.5 ± 0.9^{c}	2.5.± 0.5^{b}	0.0 ± 0.0^{d}
7.0	1.0	2.7± 0.5^{b}	1.5± 0.5^{c}	2.2± 0.8^{c}	1.0 ± 0.8^{c}
7.0	1.5	2.1± 0.3^{c}	1.1± 0.3^{c}	1.1± 0.4^{c}	0.0 ± 0.0^{d}
7.0	2.0	2.3± 0.5^{c}	0.3± 0.5^{c}	2.0± 0.3^{c}	1.0± 0.3^{c}
7.0	2.5	1.9± 0.2^{c}	0.9± 0.2^{c}	2.5± 0.4^{b}	0.0± 0.0^{d}
7.5	0.5	6.0± 0.1^{a}	0.0 ± 0.0^{d}	5.0± 0.2^{a}	0.0± 0.0^{d}
7.5	1.0	2.8±0.1^{b}	0.8± 0.1^{c}	2.2± 0.8^{c}	0.0 ± 0.0^{d}
7.5	1.5	3.0 ± 0.3^{ab}	0.0 ± 0.0^{d}	2.5± 0.1^{b}	1.0 ± 0.1^{c}
7.5	2.0	3.0 ± 0.3^{ab}	1.0 ± 0.3^{c}	2.8± 0.2^{b}	1.0 ± 0.2^{c}
7.5	2.5	2.9± 0.3^{b}	0.9± 0.3^{c}	2.5± 0.1^{b}	0.8 ± 0.1^{c}
8.0	0.5	2.0± 0.2^{c}	0.0± 0.0^{d}	1.8± 0.6^{c}	1.4± 0.6^{c}
8.0	1.0	2.2± 0.2^{c}	0.2± 0.2^{c}	2.0± 0.3^{c}	1.0± 0.3^{c}
8.0	1.5	1.2± 0.8^{c}	0.2± 0.8^{c}	1.1± 0.1^{c}	0.1± 0.1^{c}
8.0	2.0	1.8± 0.6^{c}	0.8± 0.6^{c}	1.0± 0.7^{c}	0.2± 0.1^{c}
8.0	2.5	1.0± 0.8^{c}	0.0± 0.0^{d}	0.9± 0.2^{c}	0.1± 0.2^{c}

Source: (FikaduKumsa Gemechu and Tileye Feyissa, 2016 article)

4. Conclusion

The incidence of *in vitro* shoot vetrification /hyperhydricity/ in grapevine was assesed under different concentrations of BAP and agar. From the tested different concentrations of BAP and agar, the best mean number of normal shoots was obtained at 0.5 mg/l BAP in 7.5g/l gelling agent for canonannon and cheninblanc cultivars. On other hand, the affective of growth regulators had been identified for Soltanin' and 'Sahebi' cultivars

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